



Ph. Eur. Monograph 0923: Azithromycin Related Substances on Gemini™ NX-C18 and Durashell C18(L) Columns

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Overview

Azithromycin is a broad-spectrum macrolide antibiotic which is commonly used for the treatment of bacterial infections. In this application note we show the separation of Azithromycin from its related substances following Ph. Eur. Monograph 1649. A Gemini 5 µm NX-C18 column and a Durashell 5µm C18(L) column were used and compared to the Waters® XTerra® MS C18, originally used in the monograph. To decrease the run time and improve efficiency upon the Ph. Eur. Method for the analysis of Azithromycin, an optimized method that fell within the Ph. Eur. Allowable adjustments for this gradient method was designed. Gemini 5 µm NX-C18 and Durashell 5 µm C18(L) columns were used to demonstrate the standard Azithromycin Ph. Eur. Method, while Gemini 3 µm NX-C18 column was used for the adjusted method conditions.

The use of a 150 x 4.6 mm, 3 µm of the Gemini NX-C18 column is a new (European Pharmacopoeia 11.0) allowable adjustment in a gradient method to a 250 x 4.6 mm, 5 µm since the L/dp ratio (150 mm / 3 µm = 50,000) is within the allowable range of -25 to +50 % of L/dp ratio (250 mm / 5 µm = 50,000) for the original 5 µm column used to elucidate the related substances method. The gradient and flow rate were calculated as recommended by Ph. Eur. Chapter 2.2.46 (revised July 2022) for the 150 x 4.6 mm, 3 µm column.

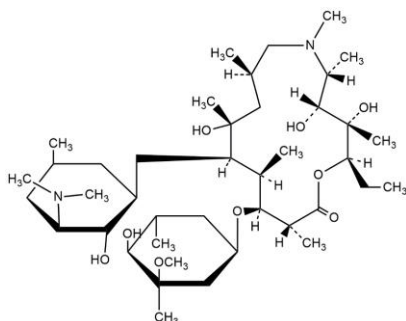
The columns used under the published monograph conditions met and surpassed the system suitability requirement for separation between Impurity F and Impurity J of a minimum for H_p/H_x of 1.4, where H_p = height above the baseline of the peak for impurity J, and H_x = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F. Relative to the XTerra MS 5 µm C18 column, the Durashell 5 µm C18(L) and Gemini 5 µm NX-C18 column gave similar separation.

The results show that both the Gemini 5 µm NX-C18 and Durashell 5 µm C18(L) columns are suitable under the conditions outlined in the Ph. Eur. monograph for Azithromycin. A baseline resolution was achieved between Impurity F and Impurity J in Reference Solution (b) for the Gemini 5 µm NX-C18, Durashell 5 µm C18(L), and XTerra MS 5 µm C18 columns. Therefore, a H_p/H_x value could not be calculated, and resolution between the two peaks is reported instead. The H_p/H_x ratio is used because baseline resolution is not typically achieved between Impurity F and Impurity J, and a resolution value can therefore not be reported. With the Gemini 3 µm NX-C18, 150 x 4.6 mm column we demonstrated a reduction in total analysis time by 64 % (from 93 min to 34 min), by implementing the changes to the gradient and flow rate as recommended by the allowable adjustments recommended in Ph. Eur. Chapter 2.2.46.

All the reference solutions were prepared as indicated in Ph. Eur. monograph 1649 for Azithromycin. The following certified reference standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: 7 Allee Kastner CS 30026 F - 67081 Strasbourg (France):

- Y0000637, Azithromycin for Peak Identification CRS
- Y0000642, Azithromycin for System Suitability CRS
- Y0000306, Azithromycin CRS

Figure 1. Azithromycin Structure



LC-UV Conditions – Original Method

Columns: Gemini 5 µm NX-C18 (00G-4454-E0)
Durashell 5 µm C18(L) (DC952505-L)
Waters XTerra 5 µm MS C18

Dimensions: 250 x 4.6 mm

Mobile Phase: Mobile Phase (Table 1)

Gradient:	Time (min)	%B
	0	50
	25	55
	30	60
	80	75
	81	50
	93	50

Flow Rate: 1.0 mL/min

Injection: 50 µL

Temperature: 60 °C

Detector: UV @ 210 nm

System: Waters Arc® HPLC

LC-UV Conditions – Adjusted Method

Columns: Gemini 3 µm NX-C18 (00F-4453-E0)

Dimensions: 150 x 4.6 mm

Mobile Phase: Mobile Phase (Table 1)

Gradient:	Time (min)	%B
	0	50
	9	55
	10.8	60
	28.8	75
	29.16	50
	33.48	50

Flow Rate: 1.667 mL/min

Injection: 30 µL

Temperature: 60 °C

Detector: UV @ 210 nm

System: Waters Arc HPLC

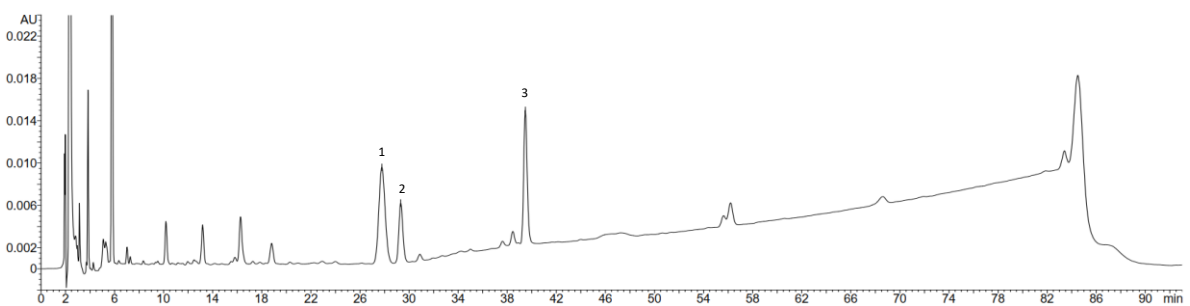
Table 1. Preparation of Test and Reference Solutions

Solution	Composition
Mobile Phase	A: 1.80 g/L solution of Anhydrous Disodium Hydrogen Phosphate R adjusted to pH 8.9 with dilute Phosphoric Acid R or with dilute Sodium Hydroxide solution R.
Diluent Buffer	B: Methanol R1 / Acetonitrile R1 (25:75, v/v). Prepare a 1.73 g/L solution of Ammonium Dihydrogen Phosphate R adjusted to pH 10.0 with Ammonia R.
Diluent	Diluent Buffer / Acetonitrile R1 / Methanol R1 (35:30:35, v/v/v).
Test Solution	Dissolve 0.200 g of Azithromycin CRS in Diluent and dilute to 25.0 mL with Diluent .
Reference Solution (a)	Dilute 1.0 mL of Test Solution to 100.0 mL with Diluent .
Reference Solution (b)	Dissolve the contents of a vial of Azithromycin for System Suitability CRS (containing impurities F, H and J) in 1.0 mL of the Diluent and sonicate for 5 min.
Reference Solution (c)	Dissolve 8.0 mg of Azithromycin for Peak Identification CRS (containing impurities A, B, C, E, F, G, I, J, L, M, N, O and P) in 1.0 mL of Diluent .



Figure 1. Related Substances Analysis Reference Solution (b)

Gemini™ 5 µm NX-C18 Column

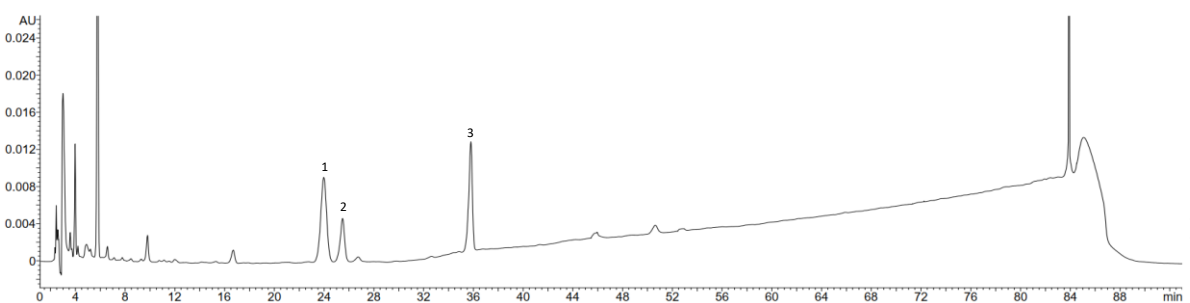


App ID 27297

Peak No.326896	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	H _p /H _v	Resolution
1	Impurity F	27.78	326896	9103	1.08	Undefined	2.0
2	Impurity J	29.32	129182	5650	1.11		
3	Impurity H	39.48	250292	12596	1.15		

N = 3 Injections

Durashell 5 µm C18(L) Column

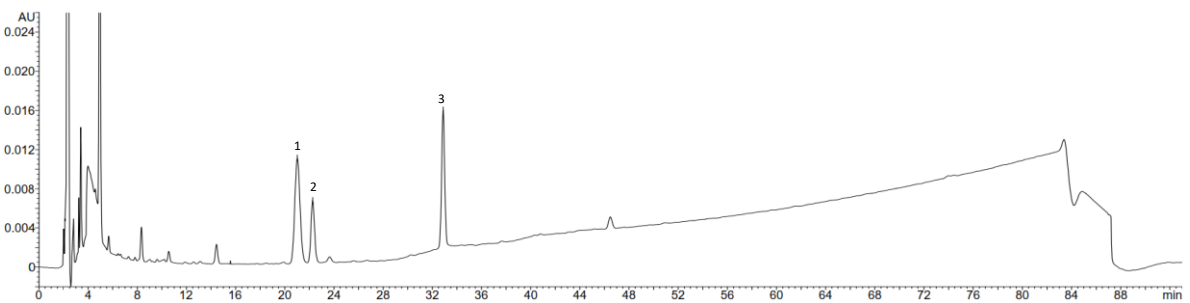


App ID 27271

Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	H _p /H _v	Resolution
1	Impurity F	23.97	301317	8701	0.94	Undefined	2.0
2	Impurity J	25.48	112599	4210	0.88		
3	Impurity H	35.80	238913	10204	0.84		

N = 3 Injections

XTerra® 5 µm MS C18 Column



App ID 27272

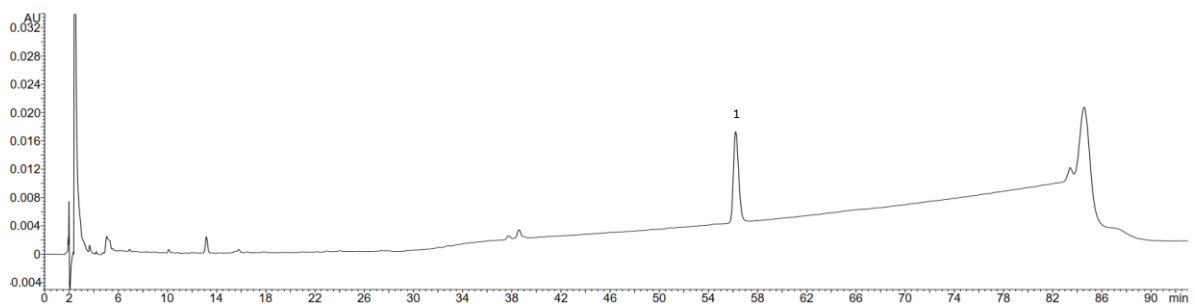
Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	H _p /H _v	Resolution
1	Impurity F	20.71	321082	10738	1.07	Undefined	2.0
2	Impurity J	22.03	129352	6353	1.11		
3	Impurity H	32.88	241592	13971	1.00		

N = 3 Injections



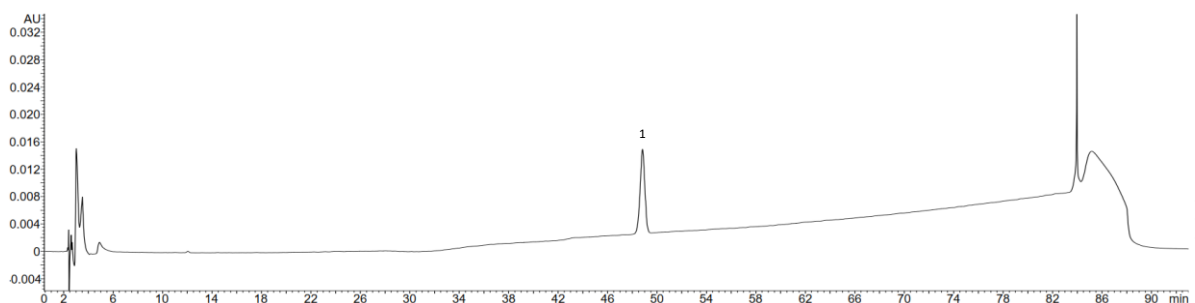
Figure 3. Related Substances Analysis Reference Solution (a)

Gemini™ 5 µm NX-C18 Column



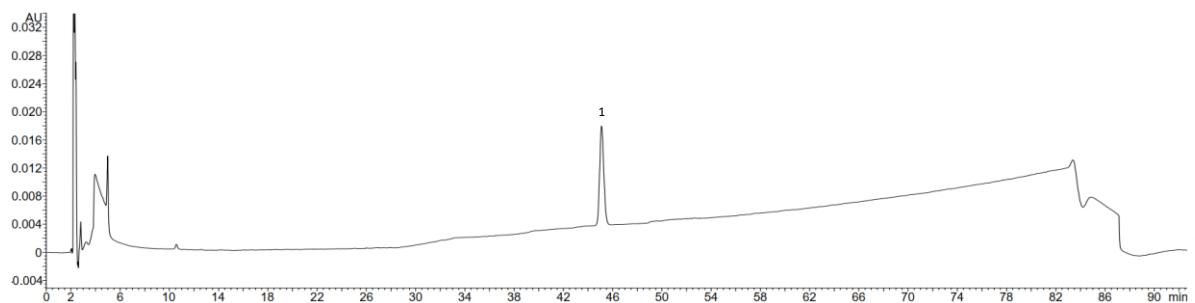
Peak No. 326896	Analyte	Retention Time (min)	Area	Height	Symmetry Factor
1	Azithromycin	56.22	376622	12810	1.22
N = 6 Injections					

Durashell 5 µm C18(L) Column



Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor
1	Azithromycin	48.82	327614	12234	0.95
N = 6 Injections					

XTerra® 5 µm MS C18 Column

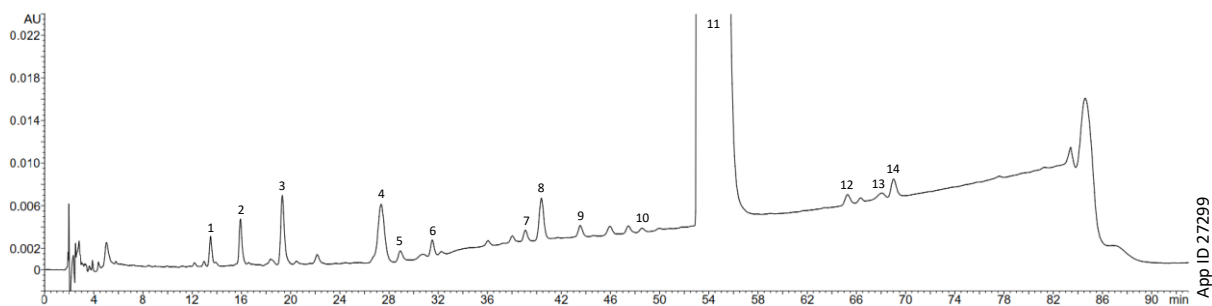


Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor
1	Azithromycin	45.10	331811	14024	1.13
N = 6 Injections					



Figure 4. Related Substances Analysis Reference Solution (c)

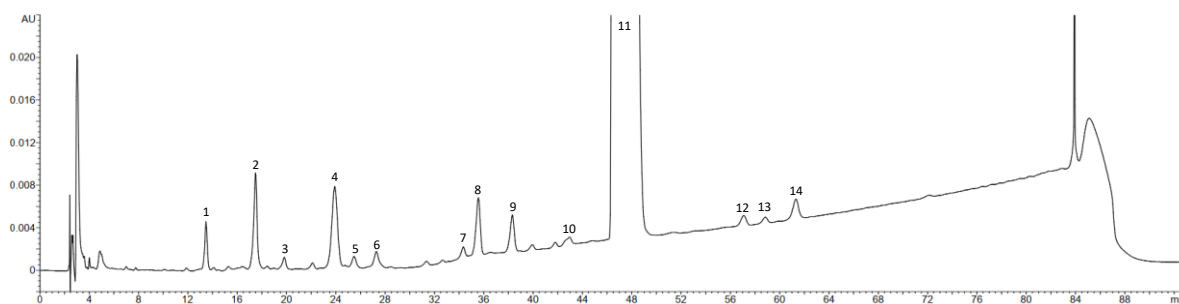
Gemini™ 5 µm NX-C18 Column



App ID 27299

Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor
1	Impurity L	13.49	35997	2654	1.06	8	Impurity N	40.39	98387	3862	1.06
2	Impurity M	15.92	66126	4204	1.33	9	Impurity A	43.54	25649	1054	1.14
3	Impurity E	19.32	133538	6454	1.42	10	Impurity P	48.55	9785	387	1.11
4	Impurity F	27.36	207053	5407	0.94	11	Azithromycin	53.24	31502309	347221	5.09
5	Impurity J	28.92	26093	994	1.16	12	Impurity O	65.27	27489	960	1.04
6	Impurity I	31.51	28189	1454	1.09	13	Impurity G	68.05	18545	500	0.78
7	Impurity C	39.08	22691	1036	1.12	14	Impurity B	69.02	47897	1657	1.17

Durashell 5 µm C18(L) Column



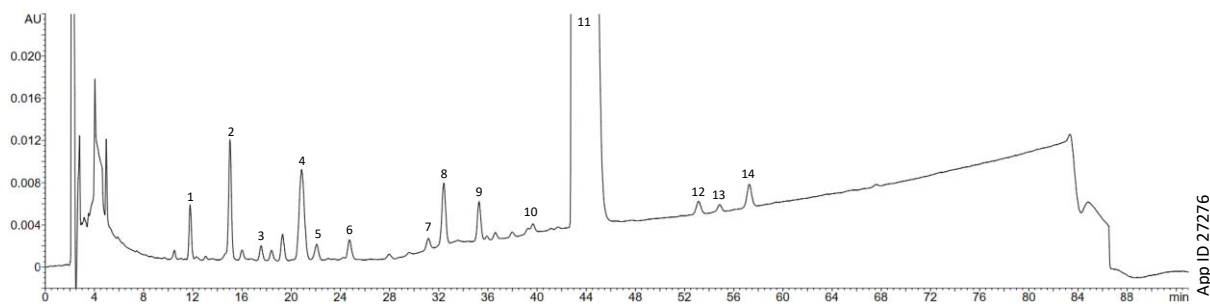
App ID 27275

Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor
1	Impurity L	13.47	65006	4501	0.95	8	Impurity N	35.56	125103	5337	0.87
2	Impurity M	17.49	177207	9006	0.91	9	Impurity A	38.31	80042	3434	0.90
3	Impurity E	19.82	24983	1142	0.88	10	Impurity P	42.96	8056	430	0.98
4	Impurity F	23.91	242104	7547	0.92	11	Azithromycin	46.50	31348153	445184	6.17
5	Impurity J	25.49	23922	1020	1.05	12	Impurity O	57.09	29732	976	0.86
6	Impurity I	27.30	39111	1448	1.20	13	Impurity G	58.84	16137	576	0.81
7	Impurity C	34.35	20898	993	0.78	14	Impurity B	61.31	64709	1890	0.82



Figure 4 Cont'd. Related Substances Analysis Reference Solution (c)

XTerra® 5 µm MS C18 Column

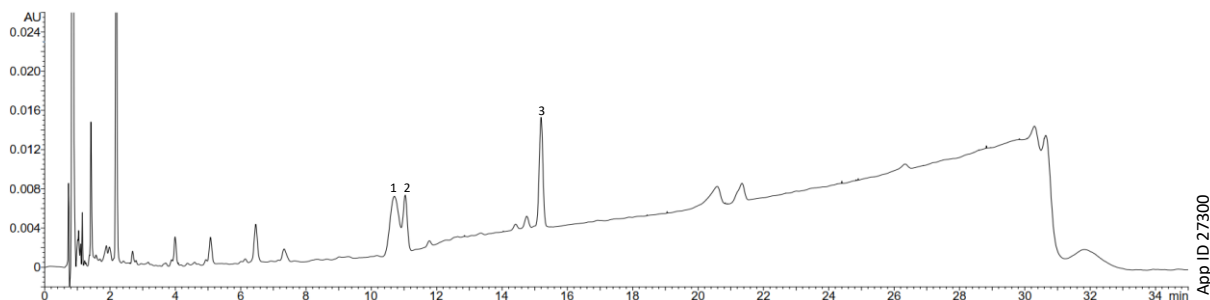


App ID 27276

Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor
1	Impurity L	11.78	67136	5086	1.13	8	Impurity N	32.38	124632	5759	0.96
2	Impurity M	15.00	165277	11008	1.08	9	Impurity A	35.24	33246	3533	1.00
3	Impurity E	17.538	23815	1414	1.06	10	Impurity P	39.66	9956	576	1.09
4	Impurity F	20.813	248780	8497	1.08	11	Azithromycin	42.86	31681423	446080	7.45
5	Impurity J	22.040	31183	1462	0.91	12	Impurity O	53.08	30859	1191	0.99
6	Impurity I	24.719	37962	1778	1.18	13	Impurity G	54.78	15919	691	0.93
7	Impurity C	31.101	23412	1091	1.02	14	Impurity B	57.21	65161	2126	1.03

Figure 5. Related Substances Analysis Reference Solution (b) – Adjusted Method

Gemini™ 3 µm NX-C18 Column



App ID 27300

Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	H _w /H _s
1	Impurity F	10.70	109558	5921	-	2.26
2	Impurity J	11.04	55195	5808	-	
3	Impurity H	15.20	87685	11129	1.0	-

N = 3 Injections



Figure 6. Related Substances Analysis Reference Solution (a) – Adjusted Method

Gemini™ 3 µm NX-C18 Column

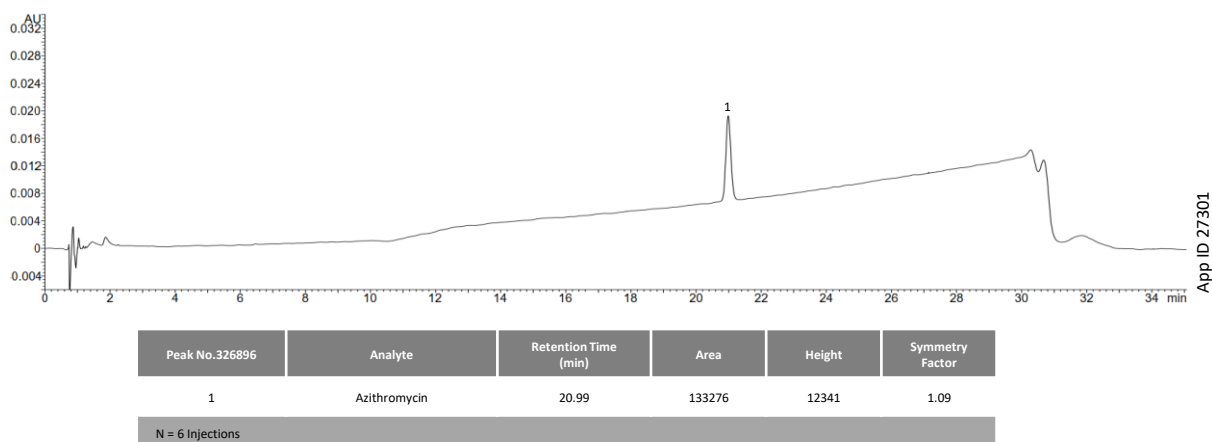
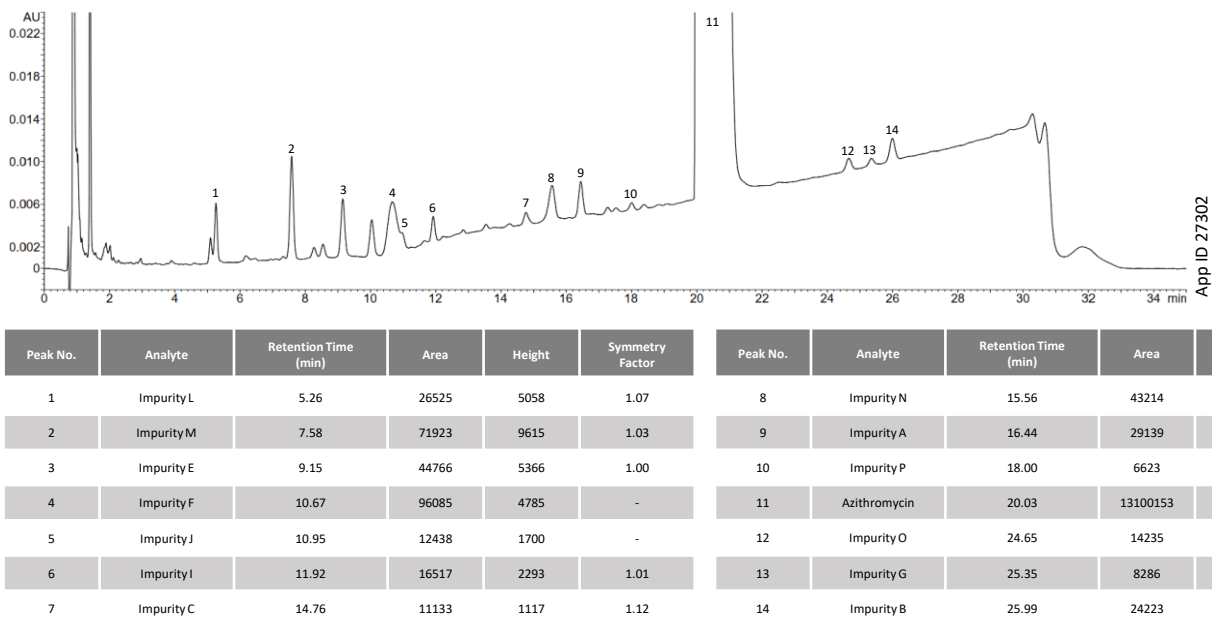


Figure 7. Related Substances Analysis Reference Solution (c) – Adjusted Method

Gemini 3 µm NX-C18 Column



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